A Randomized Pilot Trial of Dietary Modification for the Chemoprevention of Noninvasive Bladder Cancer: The Dietary Intervention in Bladder Cancer Study

J. Kellogg Parsons1,2, John P. Pierce3, Loki Natarajan3,4, Vicky A. Newman3, Leslie Barbier3, James Mohler5, Cheryl L. Rock3, Dennis D. Heath3, Khurshid Guru5, Michael B. Jameson7, Hongying Li3,4, Hossein Mirheydar1,2, Michael A. Holmes7, and James Marshall6

Abstract

Epidemiological data suggest robust associations of high vegetable intake with decreased risks of bladder cancer incidence and mortality, but translational prevention studies have yet to be conducted. We designed and tested a novel intervention to increase vegetable intake in patients with noninvasive bladder cancer. We randomized 48 patients aged 50 to 80 years with biopsy-proven noninvasive (Ta, T1, or carcinoma in situ) urothelial cell carcinoma to telephone- and Skype-based dietary counseling or a control condition that provided print materials only. The intervention behavioral goals promoted seven daily vegetable servings, with at least two of these as cruciferous vegetables. Outcome variables were self-reported diet and plasma carotenoid and 24-hour urinary isothiocyanate (ITC) concentrations. We used two-sample t tests to assess between-group differences at 6-month follow-up. After 6 months, intervention patients had higher daily intakes of vegetable juice (\( P = 0.02 \)), total vegetables (\( P = 0.02 \)), and cruciferous vegetables (\( P = 0.07 \)); lower daily intakes of energy (\( P = 0.007 \)), fat (\( P = 0.002 \)) and energy from fat (\( P = 0.06 \)); and higher plasma \( \alpha \)-carotene concentrations (\( P = 0.03 \)). Self-reported cruciferous vegetable intake correlated with urinary ITC concentrations at baseline (\( P < 0.001 \)) and at 6 months (\( P = 0.03 \)). Although urinary ITC concentrations increased in the intervention group and decreased in the control group, these changes did not attain between-group significance (\( P = 0.32 \)). In patients with noninvasive bladder cancer, our novel intervention induced diet changes associated with protective effects against bladder cancer. These data show the feasibility of implementing therapeutic dietary modifications to prevent recurrent and progressive bladder cancer. Cancer Prev Res; 6(9); 971–8. ©2013 AACR.

Introduction

In the United States in 2012, there were an estimated 73,510 new cases of and 14,480 deaths from bladder cancer (1). The U.S. population prevalence is approximately 600,000 persons and continues to increase annually (2). Bladder cancer is the fourth most frequently diagnosed cancer among men and—due to the high costs of diagnosis, treatment, and posttreatment surveillance—the single most expensive cancer to treat (3). Collectively, these observations underscore the considerable challenges bladder cancer poses to the public health and highlight an important need to develop innovative, novel therapies for bladder cancer prevention and control.

A potential means of decreasing the morbidity and mortality of bladder cancer is through lifestyle change. Modifiable risk factors present novel, practical targets for primary and tertiary bladder cancer chemoprevention because modifications of these factors potentially exert beneficial, diseasespecific health effects. For example, smoking is strongly associated with an increased risk of incident bladder cancer, and a recent cohort analysis of patients with noninvasive bladder cancer observed that longer-term smoking cessation was associated with reduced risks of disease recurrence and progression of 34% and 58%, respectively (4).

Robust epidemiological data indicate beneficial associations of increased vegetable intake, particularly cruciferous vegetables, with decreased risks of incident and progressive bladder cancer (5). In the Health Professional’s Follow-Up Study, those in the highest quartile of cruciferous vegetable intake had a 50% reduced risk of urothelial cancer compared to those in the lowest quartile (6). In a cohort of bladder cancer patients, increased consumption of raw
broccoli was associated with a 43% decreased risk of death from bladder cancer (7). Other population-based studies have observed similar patterns (8, 9).

Translational studies of lifestyle modifications and bladder cancer, however, have yet to be conducted. In a randomized clinical trial, we tested a novel intervention to increase vegetable intake in patients with noninvasive bladder cancer.

Materials and Methods

Study population

We recruited 48 patients aged 50 to 80 years at 4 study sites (Moore Comprehensive Cancer Center, University of California San Diego and San Diego Veterans Affairs Medical Center, La Jolla, CA; Roswell Park Cancer Institute, Buffalo, NY; and Waikato Hospital, Hamilton, New Zealand) with biopsy-proven noninvasive (Ta, T1, or carcinoma in-situ) urothelial cell carcinoma with at least a 3-year life expectancy and a willingness to be randomized to receive information about diet or to participate in dietary intervention. Institutional Review Board approval was obtained at all sites.

Exclusion criteria included psychiatric illness precluding compliance with the intervention and/or obtained of informed consent; medical conditions which in the opinion of the treating physician made the protocol unreasonably hazardous, including infection, chronic diseases (such as diabetes mellitus, cardiac disease, ulcerative colitis, and Crohn's disease); intolerance of cruciferous vegetables; bladder cancer with distant metastases; prior cystectomy; and unwillingness to adopt a vegetable-rich diet.

Intervention: telephone- and Skype-based dietary counseling

Patients were randomized to 6 months of telephone- or Skype-based (for New Zealand patients, n = 1) dietary counseling or a control condition that provided print materials only.

The principle strategy to promote dietary change in the intervention arm was a counseling protocol with individualized, one-on-one assistance tailored to each participant. The counseling protocol followed a step-wise, phased approach using social cognitive theory (10, 11). Motivational interviewing techniques were used to help participants assume and maintain responsibility for their own behavior change (12). Similar to a prior study we conducted in prostate cancer patients (13), we included 12 telephone calls over the 6-month intervention, with more frequent calls occurring during the early phase of the intervention when participants required more support in making dietary change. The protocol specified 5 calls during month one and 3 calls during month 2, followed by monthly maintenance calls during months 3 to 6.

The primary intervention behavioral goal was 7 daily vegetable servings, with at least 2 of these as cruciferous vegetables. We defined a serving size as half cup cut-up, chopped, or shredded vegetables; half cup vegetable sauce or puree; 1 cup of raw, leafy vegetables; or three-fourth cup (6 fl. oz.) vegetable juice. Within the context of these overall targets, participants were guided to obtain an adequate intake of all essential nutrients. To enhance quality control, all counseling was conducted centrally from the Moores UCSD Cancer Center. Before beginning counseling, counselors completed an intensive 80-hour training program; moreover, counselor performance was monitored throughout the study to ensure counselor consistency and quality.

The control group was provided the Dietary Guidelines for Americans, 2005, which recommended 5 daily servings of vegetables daily and did not emphasize cruciferous vegetables (14).

Outcome variables

Diets were evaluated at baseline and again at 6-month follow-up by a series of 3 separate 24-hour dietary recalls collected interactively via telephone interview. Each set of recalls included 2 weekdays and 1 weekend day during a 2-week period to provide data on average intake over that time period. To reduce the potential for reporting bias, the assessors were blinded to the randomization allocation. Dietary data were collected and analyzed utilizing Minnesota Nutrition Data System (NDS) software (Nutrition Coordinating Center, University of Minnesota, MN).

Plasma carotenoids are established biomarkers of vegetable intake (13, 15). Fasting blood samples were collected at baseline and at 6-month follow-up and, using high performance liquid chromatography (HPLC), analyzed for lutein, cryptoxanthin, lycopene, α-carotene, and β-carotene concentrations, which account for >90% of carotenoids in the circulation (15).

Urinary isothiocyanate (ITC) concentrations are indicators of cruciferous vegetable intake (16). Twenty-four-hour urine samples were collected at baseline and at 6-month follow-up and analyzed for cumulative urinary ITC concentrations with HPLC methodology using the cyclodensation reaction (16–18).

All laboratory analyses were conducted in the Moores UCSD Comprehensive Cancer Center Nutrition Shared Resource Laboratory, which participates in the National Institute of Standards and Technology (NIST), U.S. Department of Commerce, Micronutrients Measurement Quality Assurance (QA) Programs, and College of American Pathologists QA Program. Blood and urine samples collected at the San Diego VA, Roswell Park Cancer Institute, and Waikato Hospital were stored at −80°C until shipment to UCSD.

Statistical analysis

The study was designed to have >80% power to detect a moderate to large 0.7 effect-size (i.e., mean difference in diet change between arms divided by the SD of change) with 50 total participants, based on a one-sided t test with α = 0.05. The primary outcomes were changes in objective dietary biomarkers, namely α-carotene and urinary isothiocyanates, because vegetables, especially cruciferous vegetables, were a primary target of the intervention. Secondary outcomes were self-reported dietary intake of vegetables, fruits,
grains, and fat. For this pilot feasibility study, no multiple comparisons adjustment for sample size were made for the multiple outcomes.

We randomized participants in a 3:2 fashion to the dietary intervention (N = 30) or control arm (N = 18) using a block design. We randomized a larger proportion to the intervention arm to specifically and intensively investigate the feasibility of administering the behavior intervention in this pilot trial and to obtain estimates of effect-size and precision for planning a larger trial.

To test if randomization achieved comparable groups at baseline, we compared diet groups on baseline participant characteristics using two-sample tests. To examine dietary changes, we used two-sample nonparametric Wilcoxon tests to assess between-group differences from baseline to 6-month follow-up. We evaluated self-reported dietary values and biomarkers.

We conducted a sensitivity analysis to examine the robustness of results to missing data by fitting a linear mixed-effects model with dietary intake at baseline and 6 months as repeated outcome measures (19). We included a person-specific random effect to model person–person variability in intake and diet group, time (0, 6months), and the group × time interaction as fixed effects in the models. A significant diet × group interaction would indicate that dietary changes differed significantly between study arms. We transformed biomarker values to better approximate a Gaussian distribution in these models. We used a 5% significance level for all analyses.

Results

Baseline characteristics
The groups did not differ significantly in age, BMI, gender, ethnicity, education, time from diagnosis to randomization, or tumor stage (Table 1).

At 6-month follow-up, 83% of the participants completed the diet recall assessments (Table 2) and 81% (Table 3) provided blood and urine samples to complete the biomarker assays. Six (20%) participants in the intervention arm and 2 (11%) in the control arm did not complete the study (Fig. 1). The reasons for participant discontinuation were as follows: in the intervention group, 1 could not continue due to bladder cancer progression, 1 withdrew due to other medical reasons, 2 could not be contacted by phone, and 2 voluntarily withdrew for unspecified reasons; in the control group, 1 could not continue due to bladder cancer progression and 1 could not be contacted by phone.

Self-reported dietary intake
At 6-month follow-up, intervention patients reported significant increases in daily intakes of vegetable juice

<table>
<thead>
<tr>
<th>Table 1. Participant characteristics at baseline, stratified by study arm, in the Dietary Intervention in Bladder Cancer Study (DIBS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td><strong>Mean age, years (SE)</strong></td>
</tr>
<tr>
<td><strong>Mean body mass index, kg/m² (SD)</strong></td>
</tr>
<tr>
<td>Gender, %</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Ethnicity, %</td>
</tr>
<tr>
<td>Non-Hispanic White</td>
</tr>
<tr>
<td>Hispanic</td>
</tr>
<tr>
<td>Black</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Education, %</td>
</tr>
<tr>
<td>High school graduate</td>
</tr>
<tr>
<td>Some college education</td>
</tr>
<tr>
<td>College graduate</td>
</tr>
<tr>
<td>Post-college graduate</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td><strong>Mean time from diagnosis to randomization, years (SE)</strong></td>
</tr>
<tr>
<td>Tumor stage, %</td>
</tr>
<tr>
<td>Ta</td>
</tr>
<tr>
<td>T1</td>
</tr>
<tr>
<td>Carcinoma in situ</td>
</tr>
</tbody>
</table>

*aComparing control and intervention.
and total vegetables and lower daily intakes of total energy (kcal/d) and fat (g/d) compared to control patients. Although not significant at the 5% level, daily intakes of cruciferous vegetables and legumes increased and energy from fat decreased in the intervention compared to the control groups (Table 2).

<table>
<thead>
<tr>
<th>Table 2. Dietary composition comparing intervention to control at baseline and 6-month follow-up in the Dietary Intervention in Bladder Cancer Study (DIBS)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td><strong>N = 18</strong></td>
</tr>
<tr>
<td>Energy (kcal/day)</td>
</tr>
<tr>
<td>Fat (g/day)</td>
</tr>
<tr>
<td>Vegetable juice (servings/day)</td>
</tr>
<tr>
<td>Total vegetables (servings/day)</td>
</tr>
<tr>
<td>Cruciferous vegetables (servings/day)</td>
</tr>
<tr>
<td>Total fruit (servings/day)</td>
</tr>
<tr>
<td>Refined grain products (servings/day)</td>
</tr>
<tr>
<td>Whole grain products (servings/day)</td>
</tr>
<tr>
<td>Legumes, total (servings/day)</td>
</tr>
<tr>
<td>Legumes, soy (servings/day)</td>
</tr>
<tr>
<td>Energy from fat (%)</td>
</tr>
</tbody>
</table>

*P-value based on Wilcoxon rank sum test comparing changes in each marker between groups.
*Within group change P-value < 0.1.
*Between group difference at baseline Wilcoxon test P-value < 0.1.
*Within group change P-value < 0.05.
*Between group difference at baseline Wilcoxon test P-value < 0.05.

Dietary biomarkers
At 6-month follow-up, intervention patients showed significant increases from baseline in plasma α-carotene concentrations compared to controls. Although intervention patients also had increases from baseline in plasma lycopene, β-carotene, and total carotenoids compared to

<table>
<thead>
<tr>
<th>Table 3. Dietary biomarkers comparing intervention to control at baseline and 6-month follow-up in the Dietary Intervention in Bladder Cancer Study (DIBS)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td><strong>N = 18</strong></td>
</tr>
<tr>
<td>Lutein plus zeaxanthin (μmol/L)b</td>
</tr>
<tr>
<td>Cryptoxanthin (μmol/L)b</td>
</tr>
<tr>
<td>Lycopene (μmol/L)b</td>
</tr>
<tr>
<td>Alpha-carotene (μmol/L)b</td>
</tr>
<tr>
<td>Beta-carotene (μmol/L)b</td>
</tr>
<tr>
<td>Total carotenoids (μmol/L)b</td>
</tr>
<tr>
<td>Isothiocyanate (μmol/L)a</td>
</tr>
</tbody>
</table>

*P-value based on Wilcoxon rank sum test comparing changes in each marker between groups.
*Plasma.
*Between group difference at baseline Wilcoxon test P-value < 0.1.
*Within group change P-value < 0.1.
*Between group difference at baseline Wilcoxon test P-value < 0.05.
*Within group change P-value < 0.05.
*Urine.
controls, these differences did not attain significance (Table 3).

Self-reported cruciferous vegetable intake correlated significantly with 24-hour urinary ITC concentrations at both baseline (Spearman $r = 0.63$, $P = 0.005$) and 6-month follow-up (Spearman $r = 0.45$, $P = 0.03$). Although urinary ITC concentrations increased in the intervention arm and decreased in the control arm, these changes did not attain significance among the patients for whom 6-month comparison data were available (Table 3). Sensitivity analysis using mixed model yielded concordant results (Supplementary Appendix).

**Discussion**

In this randomized pilot trial in patients with noninvasive bladder cancer, our novel dietary counseling intervention significantly increased vegetable intake and plasma $\alpha$-carotene concentrations, significantly decreased fat and total energy intake, and marginally significantly increased cruciferous vegetable intake. In the intervention group, self-reported intake of cruciferous vegetables more than doubled ($P < 0.05$ for within group change and $P = 0.07$ for between group change; Table 2) and for total vegetable intake increased by more than 60% ($P < 0.05$ for within group change and $P = 0.02$ for between group change; Table 2). This trial is the first clinical study of a dietary intervention for bladder cancer and shows the feasibility of implementing therapeutic, chemopreventive lifestyle modifications in patients with bladder cancer.

The aim of this pilot study was to develop a feasible clinical intervention that produces changes in the diets of bladder cancer patients consistent with the putative benefits of prior epidemiological and preclinical data. Although the majority of prior evidence supports a role for dietary modification in primary prevention (6, 8, 9), at least one study observed a survival benefit for higher crucifer intake among bladder cancer survivors (7). Moreover, given the relatively high incidence of recurrence and progression, a compelling argument can be made in favor of the clinical relevance of tertiary prevention applications. Approximately 75% to 85% of patients with bladder cancer initially present with noninvasive disease; of these, 50% to 90% will recur or progress to invasive disease within 3 to 5 years despite aggressive local therapy (20, 21).

An additional finding was that self-reported cruciferous vegetable intake correlated with 24-hour urinary ITC concentrations. This finding is consistent with prior feeding studies in cohorts of hospitalized and nonhospitalized study participants (16, 22–26) and confirms that urinary ITC concentration is a robust biomarker for cruciferous vegetable intake in the setting of an outpatient clinical trial for cancer.

Unlike the increases in reported total and cruciferous vegetable intakes, changes in total carotenoids, lycopene, $\beta$-carotene and in the urinary ITC concentrations did not attain significance, most likely because of the relatively small sample sizes (Fig. 1). Although the changes were nonsignificant, total carotenoid and isothiocyanate concentrations in the intervention group increased whereas those in the control group decreased or remained stable, consistent with the intervention emphasis on cruciferous vegetables. Larger sample sizes would be needed to definitively show biomarker changes.

In addition, although objective biomarkers are useful metrics, they should not necessarily be regarded as a gold standard.
standard for measuring relevant intakes of nutrients in clinical trials, nor should they substitute for diet recall data. Individual variations in variables including, but not limited to, recent macronutrient intake (especially fat), BMI, and ethnicity may potentially influence serum and urinary concentrations of these biomarkers and introduce systematic biases (27). Biomarkers and dietary recall measures should thus be used as complementary metrics.

We believe these results will inform the design of a phase III trial of dietary modification to prevent recurrence and progression among patients with noninvasive bladder cancer. Specific data we will consider for trial design include the following: first, the carotenoid and ITC biomarker results, which suggest that power calculations for a phase III trial will require consideration of a larger sample size than originally anticipated; second, the adherence data, which suggest an anticipated dropout rate of up to 20% (Fig. 1); and, finally, the observation that 24-hour urinary ITC concentrations correlated with self-reported cruciferous vegetable intake, which potentially allows for greater reliance on patient self report, which would free patients of burdensome 24-hour urine collections and possibly increase adherence to follow-up.

Our intervention is practicable, demands few resource commitments on the part of the patient, and is low cost to implement among relatively large and geographically diverse study populations. The intervention uses straightforward, rational strategies adopted from social cognitive theory (10, 11) using the techniques of motivational interviewing (12). Similar interventions have produced marked forward, rational strategies adopted from social cognitive theory (10, 11) using the techniques of motivational interviewing (12). Similar interventions have produced marked adherence to follow-up.

Notably, because obesity and poor diet disproportionately affect African Americans and Hispanics (31), this intervention may be particularly relevant for addressing health care disparities for bladder cancer among underserved minorities. Compared to non-Hispanic Whites, African Americans with bladder cancer experience diminished cancer-specific and overall survival, are more likely to present with higher grade and higher stage disease, and have increased risks of adverse outcomes after cystectomy, including prolonged length of stay and death (32). Bladder cancer incidence and mortality are also higher among Hispanics compared to non-Hispanic Whites (33, 34).

Carotenoids, which occur in high concentrations in tomatoes, carrots, and other deeply pigmented vegetables, are putative anticarcinogenic agents. Prior studies have observed strong associations of higher plasma carotenoid concentrations with decreased risks of incident bladder cancer. In one analysis comparing the highest quartiles to the lowest, plasma total carotenoids (RR = 0.64), lycopene (RR = 0.94; 95% CI, 0.89–0.99), and β-cryptoxanthin (RR = 0.90; 95% CI, 0.81–1.00) were associated with decreased risks of incident disease (36).

Cruciferous vegetables—such as broccoli, kale, and radishes—are rich in ITCs. ITCs are potent inducers of phase II cytoprotective enzymes, including glutathione S-transferase (GST) and NAPDH:quinolone oxidoreductase 1 (NQO1). Cruciferous vegetables are believed to exert anticarcinogenic effects through induction of these enzymes. Several studies have observed an increased risk of urothelial carcinoma in individuals with GST and NQO1 genotypes associated with null or suboptimal phenotypes (37–40). Similarly, a case–control study reported that increased consumption of ITCs was associated with a 29% decreased risk of incident bladder cancer (41), and ingestion of ITC-rich broccoli sprout extract significantly inhibited bladder carcinogenesis in a rat model (42).

Strengths of this pilot study include its novel yet practical therapeutic strategy for bladder cancer primary or tertiary chemoprevention, randomized trial design that produced balanced study arms, 85% participant retention rate, and comprehensive dietary assessments. One potential limitation was the limited sample size, which could have reduced the study power to detect significant differences in most of the biomarkers. Another potential limitation is that it is unclear whether the observed changes in diet and plasma α-carotene will persist beyond 6 months. However, a prior study of breast cancer suggests that these changes could continue for at least 4 years after the initial application of the intervention (28). Finally, it is possible that recall bias occurred in that the intervention participants may have differentially reported consuming the recommended foods to please the interviewer. However, a prior analysis of diet change in breast cancer patients concluded that, although there is a potential increase in systematic error for diet assessment in an intervention group, the validity (i.e., correlation with "true" intake) of self-report is significantly higher during follow-up for intervention versus nonintervention participants (43). Thus, although the potential for error existed among self-reported diet in the intervention group, so did the potential for increased validity.

Conclusions

In patients with noninvasive bladder cancer, our novel intervention induced dietary changes associated with protective effects against bladder cancer. These data show the feasibility of implementing therapeutic, chemopreventive dietary modifications in bladder cancer patients and support the performance of phase III clinical trials focused on preventing incident, recurrent, and progressive disease.

Disclosure of Potential Conflicts of Interest

K. Guru is employed (other than primary affiliation; e.g., consulting) as a board member in Simulated Surgical Systems. K. Guru is also a consultant/advisory board in Simulated Surgical Systems. No potential conflicts of interest were disclosed by the other authors.
Authors' Contributions


Writing, review, and/or revision of the manuscript: J.K. Parsons, J.P. Pierce, V.A. Newman, L. Barbier, C.L. Rock, M. Jameson, H. Mirhedyar, J.R. Marshall, J. Mohler

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.K. Parsons, L. Natarajan, V.A. Newman, C.L. Rock, H. Li, J.R. Marshall

Research Administration, technical, or material support (i.e., reporting or organizing data, constructing databases): J.K. Parsons, V.A. Newman, J. Mohler

Study supervision: J.K. Parsons, V.A. Newman, L. Barbier, J. Mohler

Grant Support

This work is supported by NCI grant 1U01CA037447-25.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 13, 2013; revised June 7, 2013; accepted June 26, 2013; published OnlineFirst July 18, 2013.

References


Correction: A Randomized Pilot Trial of Dietary Modification for the Chemoprevention of Noninvasive Bladder Cancer: The Dietary Intervention in Bladder Cancer Study

In this article (Cancer Prev Res 2013;6:971-8), which published in the September 2013 issue of Cancer Prevention Research (1), the NCI grant number, U01 CA 037447-25, is incorrect. The correct NCI grant number is U10 CA 037447-25.

The online version has been corrected and no longer matches the print version. The authors regret the error.

Reference


Published OnlineFirst November 15, 2013.
doi: 10.1158/1940-6207.CAPR-13-0365
©2013 American Association for Cancer Research.